28.

Further Studies on the Light Sensitivity and Behavior of the Mexican Blind Characin.

C. M. Breder, Jr.
New York Aquarium,
&
E. B. Gresser,
New York University College of Medicine (Department of Ophthalmology).

(Plate I; Text-figures 1 & 2).

Introduction.

It has been shown by Breder & Gresser (1941) that the blind individuals of the Mexican Characin *Anoptichthys-Astyanax* series are light sensitive. This was made evident by their behavior in a gradient trough in which they showed themselves to be slightly but clearly light negative. No attempt had been made at the time that report was prepared to delimit the mechanism of this discriminatory power. The cystic form of the vestigial remnant of eye structure and its heavy investing pigment layer seemed to preclude its use as an organ of light detection, Gresser & Breder (1939) and Breder & Gresser (1941).

Experiments.

As one step toward understanding this mechanism of light perception a normal blind specimen was selected, and the vestigial eye structures were removed. Healing was rapid and satisfactory and no gross differences could be detected in its behavior.

Two tanks were employed, each with one-half light and one-half dark as described in Breder & Gresser (1941). Into one was placed a normal blind fish as a control and in the other the experimental fish. As in the earlier paper, counts were made of the frequency of appearance of the fish in the lighted halves of the tanks. The data obtained, which covered nearly a month, are given in Table I. This table indicates clearly that the experimental fish showed no bias to one compartment, the actual distribution being within 0.29 − of the expectation. The control, on the other hand, agreed well with the experiments previously reported, and fell to 13.71 + of expectation; showing that much bias to the dark half of the tank. Expressed in terms of percentage of random expectation with 100% representing pure randomness, above it light positiveness and below it light negativeness, the following figures represent the situation:

Experimental	100.58%
Control	72.58%

It is thus evident that the light detection of these fishes is lodged in some retention of function in the optical vesicle, despite its histological appearances to the contrary. The test fish, at this writing, is still living and apparently not in the least inconvenienced by its loss of ability to detect light. It cannot be separated from the others on a basis of behavior except by some formal procedure as above outlined. In previous papers concerning the ocular morphology of these blind fish, various types of undeveloped and mal-developed eyes were shown. Based on histological appearances, very little if any function was attributed to any eye of the cystic type even if a few scattered retinal elements were present. It was understandable that eyes architecturally intact but of comparatively microphthalmic proportions could be serviceable under restricted conditions and that anophthalmic vestiges predicated complete blindness. The present study involving individuals with defective ocular structures but in which some, if sparse, rods and cones are present leads to the inescapable conclusion that there is some subservance to light sensation. However, it remains for further behavior experiments followed by studies of histological preparations to determine the least number of retinal elements necessary for light sensation.

If the percentage of light avoidance of the control be compared with those reported by Breder & Gresser (1941), it is found that the present value of 72+%, compared with 62+% of its earlier tested tank mates and with 80−% of the larger fish taken directly from La Cueva Chica, places this fish roughly half way between those two groups. In reference to the latter values the following was stated in the earlier paper. "This difference of 17% may actually be significant. The cave fish were larger than the others and possibly overlying tissues of greater consequent thickness may account for the difference, or it may be that there is an increased avoidance to light in subsequent generations." Since those experiments were made the fishes reared in light for five generations (62+%) have grown so as to just about half close the size difference between them and the individuals from the cave (80−%). Consequently the lessened avoidance to light is about proportional to their growth (72+%). This suggests strongly that of the two quoted alternatives the first would seem to be the true one. In other words, it seems clearly indicated that the amount of overlying tissue determines to a considerable extent the reactivity of these fish to light. Further studies on much smaller specimens would go far to establish this view.

In order to better understand the normal unrestricted behavior of these fishes, an out-door pool was established in which approximately one-half was darkened so as to form a "cave" into which entry could be made from under water only. The entrance was provided with a light trap so that a true cave-like darkness could be obtained. This portion of the pool was roofed over and buried under about six inches of soil in which trailing vines were planted. The general arrangement and details of construction are given in Text-figure 2 while Plate I shows how the finished structure appeared.

Both blind "5th generation" and tank-raised normal river fish were placed in this pool and allowed to act according to their normal instincts. The trap door in the roof of the "cave" was padlocked and not opened until the termination of the experiments. Food was admitted to the "cave" through a small hole which was otherwise kept corked. More food was placed in the cave than in the lighted portion in order to prevent any possible bias to the outside based on hunger. Water was introduced only to make up for evaporation. This drained from another pool into the darkened portion through a light trap of loose rocks and gravel. From here it flowed through the cave and out into the lighted portion, simulating so far as possible the conditions in La Cueva Chica (see Bridges, 1940). Water lillies and *Nitella* grew in the lighted portion. In this brightly sunlit pool shade was provided by an offset in the cave wall from which the lillies grew and which was further shaded by a large *Cyperus* plant so that fishes would not necessarily be driven into the cave for the mere want of shade. As noted above, the water used drained from another well-seasoned pool which was being used for other fishes. This pool, shown in connection with other matters by Breder (1939 and 1940) and described in detail therein, drained into the new blind fish pool from its northeast corner.

TABLE I.

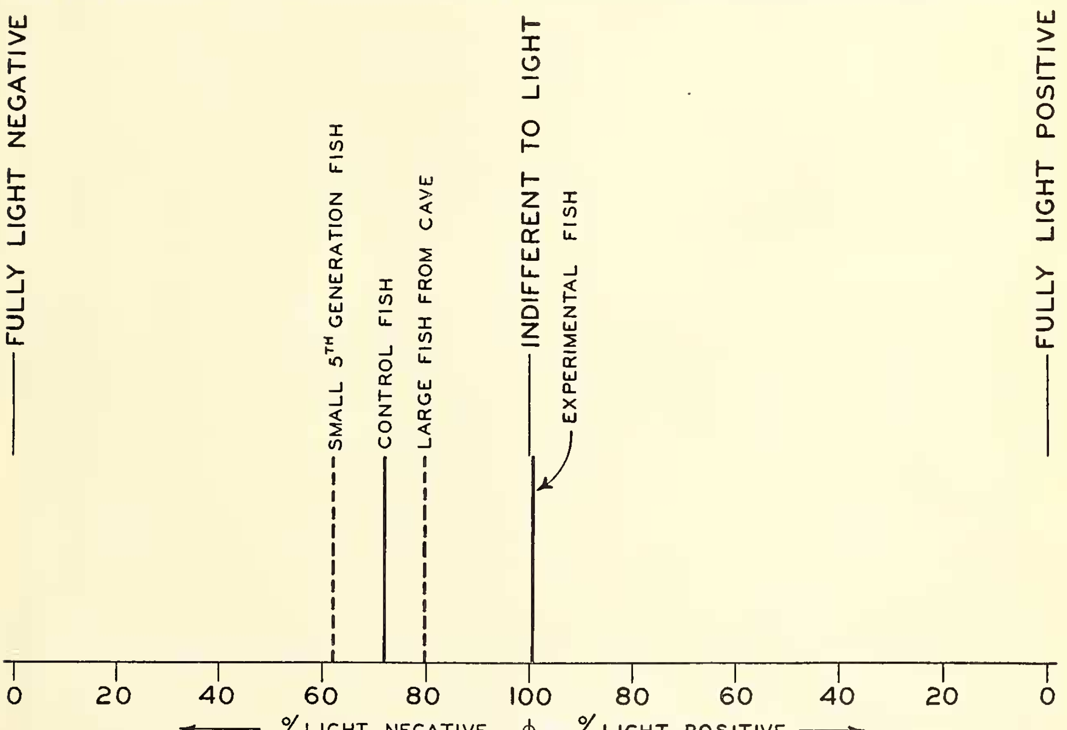
Exp. No.	Date 1941	Hour	Number of observations in light: Fish No. 33	Fish No. 34
1	5/28	4:00 p.m.	48	32
2	5/29	9:30 a.m.	47	40*
3	5/29	4:30 p.m.	51	20
4	6/2	10:40 a.m.	36	39
5	6/2	4:30 p.m.	54	19
6	6/3	9:00 a.m.	53	27
7	6/3	4:15 p.m.	8	44
8	6/3	——	32	36
9	6/4	8:40 a.m.	56	35
10	6/4	4:45 p.m.	56	36
11	6/5	9:25 a.m.	65	35
12	6/5	4:25 p.m.	57	37
13	6/6	9:30 a.m.	64	27
14	6/6	4:45 p.m.	60	46
15	6/7	10:30 a.m.	55	34
16	6/7	4:55 p.m.	58	34
17	6/8	11:15 a.m.	55	26
18	6/8	5:00 p.m.	50	29
19	6/9	10:00 a.m.	60	47
20	6/9	4:10 p.m.	50	40
21	6/10	——	36	37
22	6/10	——	58	33
23	7/1	9:45 a.m.	44	46
24	7/1	4:45 p.m.	38	34
25	7/2	10:15 a.m.	51	43
26	7/2	4:30 p.m.	39	37
27	7/3	9:45 a.m.	52	34
28	7/3	4:45 p.m.	62	50
29	7/4	11:00 a.m.	72	39
30	7/4	3:30 p.m.	56	37
31	7/5	11:00 a.m.	63	44
32	7/5	3:00 p.m.	60	57
33	7/6	11:25 a.m.	38	34
34	7/6	4:50 p.m.	32	34
35	7/7	10:20 a.m.	44	40
36	7/7	2:30 p.m.	10	41
37	7/8	10:30 a.m.	32	28
38	7/8	2:30 p.m.	23	18
39	7/9	9:00 a.m.	74	44
40	7/9	3:30 p.m.	54	40
41	7/10	9:30 a.m.	73	46
42	7/10	——	86	25
			2112	1524
		Average	50.29−	36.29−
		Maximum	65	74
		Minimum	8	10
			% of Expectation	
		Average	100.58−	72.58−
		Maximum	130	142
		Minimum	16	20

*Each experiment represents 100 notations at 5-second intervals except the figure marked which was 75 and recalculated. There were 30 notations in light of the 75. Most of the actual readings were made by Mr. Max Recher.

The latter had been originally built for another purpose, see Shlaifer & Breder (1941).

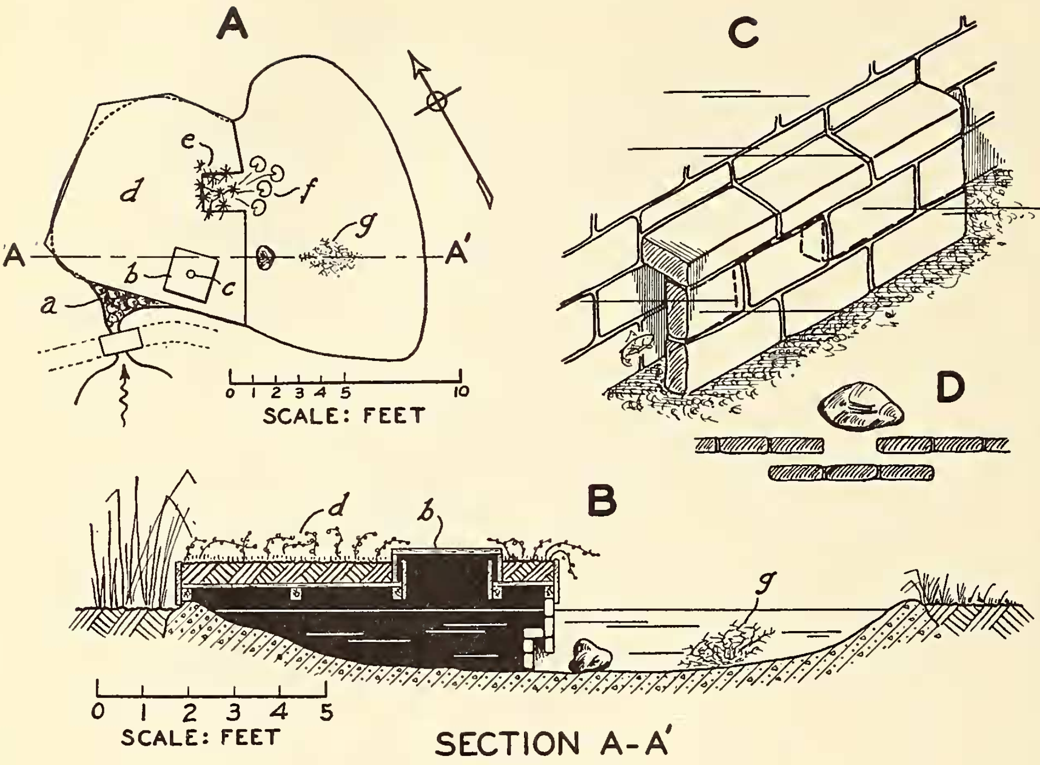
Data on temperature differentials and similar matters are given in chronological order in Table II. The fishes were all introduced into the lighted portion of the pool. Initially two blind fishes were placed in the pool to test if they could stand the early spring temperatures. In less than twenty-four hours these fish found the cave entrance and spent much time in the cave from then on, but wandered in and out at will. Apparently it took no longer time than this for them to learn their way about in the area of this pool for after the first day it was noted that they hardly ever struck objects and they would go in and out the somewhat tortuous entrance touching nothing. Since they show no evidence of distance reception, as noted by Breder & Gresser (1941) it would appear that they can satisfactorily learn the terrain of about one hundred and fifty square feet of area, which the pool covers, in that time.

In order to determine how much time was spent in and out of the cave, periods of observation were made on May 26 which cover a total observational time of three hours. The times of entrance and emergence from the cave were calculated. It was found that 83.3+% of the time was spent within the cave and 16.6+% in the lighted pool. This agrees well with the data of Breder & Gresser (1941) and that given herein in Table I. The present figure, while slightly higher than any of the others, is based on relatively few observations and has been necessarily calculated on a somewhat different basis. Further work of this nature could not be continued when more fish were introduced for a variety of purely practical reasons. Also it was subsequently found that other influences so modified this light avoidance reaction, which were not present in the laboratory work, that additional readings would have been without value. As it developed it was found that these influences could be readily understood and described but did not lend themselves easily to a statistical approach or analysis in this outdoor pool. Usually a few blind fish could be seen but there were long periods when all were in the cave and equally long ones when all were out of the cave. When the normally visioned river fishes were introduced on June 2, they also partook of this variation in habit. In their case, with marked schooling habits, it was clear that the activities of one individual had a sharp bearing on the others. Here, as in aquaria, they would attempt to school with a blind fish wandering by but soon gave up to return to their own apparently more satisfactorily behaving eyed com-



Text-figure 1.

Comparison of behavior between experimental and control specimen (shown in solid lines) together with the data of Breder & Gresser (1941) (shown in dotted lines) in terms of expectancy of random movement.



Text-figure 2.

Construction and details of experimental "cave." **A.** Plan of pool and cave as constructed. **B.** Section through pool and cave. **C.** Light trap as seen from inside cave. **D.** Plan of light trap. **a.** Loose stones and gravel to form light trap and block fish exit at point of inflow to pool. **b.** Trap door to cave. **c.** Corked hole in trap door for feeding and temperature reading. **d.** Roof of cave composed of flooring on stringers covered with soil and planted with vines. **e.** Large *Cyperus* plant. **f.** *Nymphaea.* **g.** *Nitella.*

panions. This plus the large areas involved apparently prevented the results disastrous to the blind individuals noted by Breder & Gresser (1941). At least at no time was anything seen that could have been interpreted as an attack on the blind by the normal eyed fishes. At times the addition of water would cause all to rush into the outflowing stream and enter the cave, where presumably they sought the blocked inflow from the other pool.

These effects were variable and at first puzzling but it soon appeared that the causes lie in temperature differentials between the cave and the open pool. For example there was a marked tendency for all the fishes to be out in the open in the daytime and to retreat to the cave at dusk, both eyed and blind alike. During the daytime the open pool would warm under the influence of solar radiation and rise several degrees above the cave temperature while at night it would drop below that of the cave which because of its cover did not lose its temperature nearly so fast. A study of Table II will indicate how these varying seasonal and diurnal temperature changes effected the relationships of the water temperature in the cave to that in the open pool. The results may be summarized as follows:

1. If the temperatures were identical, or nearly so, the fish would move in and out freely, usually with the majority of blind fish out of sight and the eyed specimens in a compact school in the lighted pool.

2. A difference of as little as 1.5° F. between the cave and the open pool would cause practically all to be in the warmer section, independent of time of day or brightness of light.

3. Water entering from the other pool would have various effects dependent on whether it was warmer than that in the cave or not. If the cave was notably cooler than either pool the water first passing into the lighted portion and cooling it would first prevent the fish from entering the cave and later as the cave warmed from the new water and the open pool cooled they would all enter, stimulated both by flow and temperature.

Many of both types of fish were evidently gravid during the middle of summer but it is doubtful whether any spawning took place as the

TABLE II.

Temperatures and related data.

Date	Hour	Water temperatures °F. Cave	Pool	Max.	Min.	
May 23	9:00 p.m.	—	73	—	—	2 cave fish in pool
24	7:00 p.m.	—	—	70	64	
25	7:00 p.m.	—	—	65	58	
26	10:30 a.m.	62	69	—	—	
	4:00 p.m.	60	67	—	—	
	7:00 p.m.	—	—	69	57	
29	8:00 p.m.	—	—	77	66	
30	3:00 p.m.	66	67	72	62	10 cave fish in pool
31	8:00 p.m.	—	—	74	60	
June 1	4:00 p.m.	63	62	—	—	
	6:00 p.m.	—	—	66	61	
2	8:00 p.m.	65	65	72	61	9 river fish in pool
3	8:00 p.m.	—	—	75	61	
4	6:30 p.m.	—	—	70	61	
July 14	3:00 p.m.	69	73	—	—	
18	8:00 p.m.	—	—	75	67	
20	8:30 p.m.	—	—	78	63	
22	7:45 a.m.	69	67	—	—	
	8:00 p.m.	69	73	78	62	
23	7:45 a.m.	70	69	—	—	
	8:00 p.m.	74.5	77	82	67	
24	7:45 a.m.	73	71	—	—	
	8:00 p.m.	78	76	81	68	
26	8:30 a.m.	75.5	75	—	—	
	8:00 p.m.	75.5	78	82	71	
27	10:30 a.m.	74	76	—	—	
	8:00 p.m.	77	78	82	67	
30	7:30 p.m.	72	72	78	70	
31	6:30 p.m.	72	72	71	70	
Aug. 2	9:30 a.m.	73.5	73.5	77	69	
	7:00 p.m.	74	75	79	75	
3	8:00 p.m.	72.5	73.5	79	66	
16	9:00 a.m.	66	67	83	53	
18	5:00 p.m.	64	66	73	53	
28	2:00 p.m.	65	66	78	58	
Sept. 5	9:00 a.m.	67.5	66	79	53	
8	10:00 a.m.	66	63	70	56	
	1:30 p.m.	65	66.5	—	—	
	4:00 p.m.	67	66	—	—	
13	2:00 p.m.	62	60	—	—	Removed fish, 1 cave, 4 river.
Mean		69.2 +	70.1 −	75.3 +	62.9 +	
			69.1 +	(average of all min. and max.)		
Maximum		78	78	83	75	
Minimum		60	60	65	53	

Note. Columns marked "Max. and Min." read on a minimax thermometer at hour noted and reset for next reading.

temperatures were probably below their spawning threshold. In any event there were no young evident, although either eggs or very young fish may have been eaten by the adults or by aquatic insects if such were actually produced.

The experiment was terminated with the advent of cool weather as it was evident that soon their lower threshold would be passed. As it was, some of the minimum temperatures reached and survived by these fish are rather remarkable, especially when it is borne in mind that the home waters of these fish hover about 80° F. Since the fish were variously in and out of the cave it was impossible to keep a close check on the actual numbers present. When the fish were removed on September 13 it was found that only five remained, one blind and four eyed. Less than two weeks previously many more than this number were seen when a warm day drew them out of the cave. It is suspected that with the cooling water and a failure of their alertness that they fell prey to frogs, as the most likely predator normally about these pools. No matter what the cause of their destruction, under the conditions of natural enemies, fluctuating temperatures, et cetera, it is clear that a marked differential of survival is present. Reduced to terms of percentage, 10% of the blind individuals survived while 44+% of the eyed ones were still there when the experiment was terminated. Although it must be admitted that the total numbers are small, those that were recovered were in an excellent state of vigor and are now in aquaria. We have every reason to believe that the difference in numbers has bearing on both the blindness and light color of the cave fish. Incidentally it seemed odd that the frogs did not catch the blind fish early in the experiment, for although they knew their way around the pool they could not tell where a large frog might be floating and if both were present would sooner or later bungle into the frog's dangling hind legs. At first this unaccustomed accident would cause the frog to give a typical fright reaction but soon the frogs became accustomed to such incidents and on occasion they would turn and act as though to pursue the blind fish. The safety of these fish seemed to reside in the fact that customarily when they do strike into something they ordinarily charge off into another direction at top speed, and then not infrequently seem to be confused and are likely to strike the bottom or something else with which they are fully familiar and take some little time to quiet down again.

Discussion.

Since it has been shown that reactions to temperature gradients override reactions to light in both blind and visual types it may well follow that temperature differentials in a state of nature form an important influence on the entry and further penetration of these caves. There would thus seem to be at least three factors in the behavior of these fish leading to cave entry.

1. Negative phototaxis on the part of blind fish and a tendency for visual types to hide in dark places and peer out.

2. Positive rheotaxis tending to cause these fishes to swim upstream.

3. Positive thermotaxis tending to cause these fishes to move into warmer waters.

The first two requirements of the environment are met by La Cueva Chica and while at the time of our visit the subterranean waters were practically of the same temperatures as surface waters, surely at other seasons the ground waters are warmer than those of surface drainage. During the dry season these ground waters should be cooler than these of the surface if they were not heated from below, a geologic feature of this region with its magnetic layers close to the surface and with an abundance of warm springs.

Although the temperatures encountered in the field were considerably above those obtainable in the pool experiments there is no reason to suppose that the fishes' behavior would be any different in regard to thermal preferences. Incidental to this is the fact shown by Doudoroff (1938) that a variety of marine species will select water warmer than that of their normal habitat, if given the opportunity.

This finding of a temperature gradient reaction helps to account for the presence of fishes as remote from the cave's exit as is evidently possible for them to reach, for presumably there is such a gradient from these cave springs, where they rise from the depths, to the juncture at the river, during part of the year at least, although in the short section of the cave available to human exploration no such gradient was detected. The cave water temperatures taken in a wide variety of places varied scarcely at all from 80° and this quite erratically. In aquaria, the same fish appeared to be not in the least inconvenienced by temperatures in the low ninety degrees although eggs laid in water of such temperatures failed to develop normally. Temperatures as low as 65° and for short intervals dropping to 53° have been survived. It thus appears that these fish have a rather wide temperature tolerance. Nevertheless, normally eyed river fish, hatched in an unfurnished, rectangular and well lighted aquarium with no opportunity to hide at all, voluntarily entered a simulated cave where their eyes were useless, on the stimulation of slight flow or a slight thermal difference. Blind fish of the fifth generation raised in light and without previous experience behaved the same way. Given fishes with three such items in their normal behavior, all that would seem to be necessary, in addition, to establish the observed condition in La Cueva Chica would be a genetic defect involving the eyes and cave factors of the types described.

The differential in survival under the conditions of the experiment may mean but little in terms of the Mexican caves but since the experimental temperatures drew the blind fish out of the cave nearly daily it is tempting to imagine that, had it been possible to reverse conditions at will, a much larger number of blind individuals would have survived.

Summary.

1. Light sensitivity in *Anoptichthys* is lodged in the apparently functionless remnant eye capsule, which may be demonstrated by its removal.

2. Reactions to temperature overrides the influence of light in both the eyed and blind forms and rheotaxis interferes to a lesser extent.

3. Eyed individuals apparently do not attempt to destroy blind ones except under conditions of close confinement.

4. Normal river fish, which never before had an opportunity, entered a simulated cave on temperature differentials of less than 1.5° F. as well as on a slight flow emerging from the darkness.

5. Survival in an outdoor pool divided about equally between light and dark was in favor of the eyed forms slightly better than 4 to 1.

6. Three factors have been experimentally demonstrated as parts of the normal behavior pattern of both eyed and eyeless fishes, which would tend to make them enter caves similar to the one in which found, namely: negative phototaxis, positive rheotaxis and positive thermotaxis.

Bibliography.

Breder, C. M., Jr.
1939. Variations in the nesting habits of *Ameiurus nebulosus* (LeSueur). *Zoologica* 24 (25): 367–378.

1940. The nesting behavior of *Eupomotis gibbosus* (Linnaeus) in a small pool. *Zoologica* 25 (23): 353–360.

Breder, C. M., Jr. & Gresser, E. B.
1941. Correlations between structural eye defects and behavior in the Mexican blind Characin. *Zoologica* 26 (16): 123–131.

Bridges, W.
1940. The blind fish of La Cueva Chica. *Bull. N. Y. Zool. Soc.* 43 (3): 74–97.

Doudoroff, P.
1939. Reactions of marine fishes to temperature gradients. *Biol. Bull.* 75 (3): 494–509.

Gresser, E. B. and Breder, C. M. Jr.
1939. The histology of the eye of the cave characin, *Anoptichthys*. *Zoologica* 25 (10): 113–116.

Shlaifer, A. & Breder, C. M., Jr.
1940. Social and respiratory behavior of small tarpon. *Zoologica* 25 (30): 493–512.

EXPLANATION OF THE PLATE.

Fig. 1. The pool and "cave" as it appeared with the construction work finished but before the placement of soil and planting.

Fig. 2. The pool and "cave" as it appeared shortly after planting. Later the vegetation became considerably more lush, hiding all of the woodwork.